

Short Communication

Augmented Growth Equation for Cell Wall Expansion¹

Received for publication January 8, 1985 and in revised form June 6, 1985

JOSEPH K. E. ORTEGA

Department of Mechanical Engineering, University of Colorado at Denver, Denver, Colorado 80202

ABSTRACT

The Growth Equation representing the relative rate of irreversible wall expansion is augmented with an elastic expansion component. Some of the utility of this augmented Growth Equation is demonstrated through selected applications.

The physical theory of plant cell growth suggests that the rate of cell expansion is determined by the rate of two simultaneous physical processes: water uptake and irreversible cell wall extension. Equations relating the rate of plant cell expansion to both the rate of water uptake and the rate of irreversible cell wall extension were first published by Lockhart (5), and subsequently by Ray *et al.* (7). These equations and their combination have been termed the 'Growth Equations' (8). A recent review by Taiz (8) demonstrates the general utility of the Growth Equations for understanding the role that biophysical, biomechanical, and biochemical parameters play in plant cell growth and growth regulation. Implicit in this biophysical approach is the assumption that biological and biochemical control of plant cell expansion is mediated by controlling the magnitude of the biophysical and biomechanical parameters. It is noted that the Growth Equations establish a quantitative relationship between the rate of cell expansion and a variety of biophysical and biomechanical parameters that can be measured or determined *in vivo*, and that the Growth Equations may provide the foundation for the development of a general quantitative model for plant cell growth and growth regulation.

In spite of the general success of these equations, some limitations and shortcomings have been identified. One of the limitations is that the Growth Equations are valid only for steady state growth, or more accurately, equilibrium growth. In recent years there has been an increasing need to adapt the Growth Equations to handle the period when the cell is in transition from one equilibrium state to another (1, 3).

It is the objective of this paper to augment the Growth Equation describing the rate of irreversible cell wall expansion with an 'elastic stretching' component, and to demonstrate the utility of this new equation through selected applications.

THEORY

Lockhart (5) proposed that elongation of a cylindrical plant cell wall is the sum of the change in length due to irreversible extension and the change in length due to elastic stretching. Near

the beginning of Lockhart's derivation of a relationship between the relative rate of elongation and the force, he assumed that the force, F , was constant, *i.e.* $dF/dt = 0$. Subsequently, it was further assumed that the rate of irreversible extension is a linear function of the turgor pressure, *i.e.* that the cell wall behaves as a linear viscoelastic element. In terms of total volumetric cell expansion, not just elongation, the derived relationship for the relative rate of volumetric expansion, \dot{V} , of the cell wall chamber is:

$$\dot{v} = dV/(V dt) = \theta P \quad (1)$$

where V is the volume of the cell wall chamber, θ is the extensibility of the cell wall, and P is the turgor pressure. It is noted that this equation is analogous to the constitutive relationship (between the strain rate and the stress) for a Newtonian fluid:

$$\dot{\epsilon} = \sigma/\mu \quad (2)$$

where $\dot{\epsilon}$ is the strain rate (for elongation only, $\dot{\epsilon} = dl/(l dt)$), σ is the stress, and μ is the dynamic viscosity. Now assuming that σ is directly proportional to P , and substituting, P for σ , θ for $1/\mu$, and, \dot{v} for $\dot{\epsilon}$, Eq 1 is obtained from Eq 2. Another constitutive relationship is obtained in the case where the stress must exceed some minimum value, σ_0 , (yield stress) before irreversible strain occurs:

$$\dot{\epsilon} = (\sigma - \sigma_0)/\mu \quad (3)$$

Now making the previously mentioned assumptions and substitutions, we can obtain the following:

$$\dot{v} = \theta(P - P_c) \quad (4)$$

where P_c is the critical turgor pressure, and corresponds to the yield stress, σ_0 . This is the equation for irreversible cell wall extension which was first derived for elongation only by Lockhart (5) and subsequently derived for volumetric expansion by Ray *et al.* (7).

At this point, it is important to remember that the force, and thus the stress and turgor pressure, was assumed to be constant ($dF/dt = d\sigma/dt = dP/dt = 0$). Furthermore, it is important to recognize that because of this assumption the elastic component of the total cell wall extension or expansion was eliminated from the constitutive equations (Eq 1–4). Now if Hooke's law is assumed to hold for the elastic extension, then the appropriate constitutive relationship will be similar to that of a Maxwell linear viscoelastic model:

$$\dot{\epsilon} = (\sigma - \sigma_0)/\mu + (d\sigma/dt)/E \quad (5)$$

where E is Hooke's modulus. Again making the previous assumptions and substitutions, and one other, $\epsilon = E$, then the following relationship is obtained for the relative rate of volumetric expansion of the cell wall chamber:

$$\dot{v} = \theta(P - P_c) + (dP/dt)/\epsilon \quad (6)$$

¹ Supported by Faculty Development Grant Awarded by The University of Colorado at Denver's Research Council.

where ϵ is the volumetric elastic modulus of the cell wall (4, 6). Eq 6 now contains the 'elastic stretching' component that was eliminated at the beginning of Lockhart's derivation. It should be noted that although Hooke's law was used to obtain Eq 5 and 6, this was done for the sake of simplicity and expediency. In the most general case, E and ϵ do not need to be constant for Eq 5 and 6 to be valid. In the specific case of Eq 6, ϵ can be a function of both P and V . A review of the literature suggests that ϵ is dependent on both P and V ; for nongrowing plant cells (4, 8). Although this is very suggestive for the growing plant cells, there are not any data known to this investigator which demonstrate the dependency of ϵ on P and V for growing plant cells. In fact, Eq 6 is needed to obtain the criterion for an accurate measurement of ϵ for a growing plant cell (see section C). The inclusion of the 'elastic stretching' component adds to the general utility of the Growth Equations. It is the objective of the next section of this paper to demonstrate some of the utility of this new Growth Equation (Eq 6).

APPLICATIONS

To investigate the utility of this new Growth Equation for the relative rate of volumetric expansion of the cell wall chamber (Eq 6), we will begin with two cases (A and B) where this equation reduces to forms previously used. Subsequently, we will proceed to applications of Eq 6. It will be shown that this equation will provide the criterion for an accurate measurement of the cell wall volumetric elastic modulus, ϵ , when the plant cell is growing (case C). It will also be shown that from this equation and its solution for the turgor pressure, the critical turgor pressure, P_c , can be determined (case D). Finally, it is shown that this equation can be combined with the equation representing the rate of water uptake and solved to obtain a solution for the turgor pressure behavior after instantaneous changes in the magnitude of certain biophysical and biomechanical parameters (case E).

(A) When the Turgor Pressure, P , is Constant. The condition of constant turgor pressure, where $dP/dt = 0$, is representative of equilibrium growth. Because $dP/dt = 0$, the elastic component of the relative rate of volumetric expansion drops out of Eq 6 leaving Eq 4, as first reported by Lockhart (5) and later by Ray *et al.* (7).

(B) When the Cell Wall Extensibility, θ , is Zero. The condition where the extensibility is zero, represents a living and turgid, but nongrowing plant cell. When $\theta = 0$, the irreversible wall expansion component of Eq 6 drops out, leaving the following relationship:

$$\dot{v} = dV/(V dt) = (dP/dt)/\epsilon \tag{7}$$

After multiplying through by dt and rearranging, we get:

$$\epsilon = V(dP/dV) \cong \Delta P/(\Delta V/V) \tag{8}$$

which is simply the definition of the volumetric elastic modulus (4, 6). The volumetric elastic modulus, ϵ , can be determined by incrementally increasing the turgor pressure, ΔP , and determining the respective increase in volume, ΔV (4). A plot of ΔP versus ΔV will have the slope of ϵ/V , where V is the reference volume of the cell when $\Delta P = 0$.

(C) Determination of the Volumetric Elastic Modulus, ϵ , When the Extensibility, θ , is Not Zero. The criterion for determining the volumetric elastic modulus, ϵ , for a growing plant cell (when $\theta \neq 0$) can be determined from Eq 6.

Substituting finite differences for derivatives in Eq 6, we get:

$$(\Delta V/\Delta t)/V = \theta(P - P_c) + (\Delta P/\Delta t)/\epsilon \tag{9}$$

Rearranging and solving for ϵ , we obtain:

$$\epsilon = \Delta P/[(\Delta V/V) - \theta(P - P_c)\Delta t] \tag{10}$$

Thus, it is apparent that the criterion for obtaining an accurate measurement of the volumetric elastic modulus is:

$$\theta(P - P_c)\Delta t \ll \Delta V/V \tag{11}$$

Since the time interval, Δt , used to incrementally increase the pressure (and thus the volume) is the only parameter under the experimenter's control, it can be determined how small it must be to obtain an accurate value for ϵ .

(D) Determination of the Critical Turgor Pressure, P_c . Cosgrove *et al.* (3) have experimentally demonstrated that when the growing plant cell is isolated from its water source and the transpiration from the plant cell is eliminated, the turgor pressure will begin to decay exponentially to a constant value which they reason to be the critical turgor pressure, P_c . This procedure is equivalent to a stress relaxation test in material sciences. The condition for a stress relaxation test is that the strain rate is zero ($\dot{\epsilon} = 0$). The equivalent condition for the growing plant cell would be that the relative rate of volumetric expansion is zero ($\dot{v} = 0$). Applying this condition to Eq 6, we obtain the following differential equation for a pressure relaxation test:

$$0 = \theta(P - P_c) + (dP/dt)/\epsilon \tag{12}$$

Rearranging, we obtain:

$$dP/dt + \epsilon\theta P = \epsilon\theta P_c \tag{13}$$

Now in the most general case, the magnitude of ϵ , θ , and P_c will all be functions of the turgor pressure, P , and the solution to Eq 13 will depend on the form of these functions. Presently, it is not known if ϵ (for a growing plant cell) or θ are dependent on P . Thus, only the simplest case will be considered where we assume ϵ , θ , and P_c to be a constant. Then this differential equation can be integrated with the initial condition that at $t = 0$, $P = P_i = \text{constant}$, to obtain the following solution:

$$P(t) = (P_i - P_c) \exp(-\epsilon\theta t) + P_c \tag{14}$$

where it is apparent that at $t = 0$, $P_c = P_i$, and at $t = \infty$, $P = P_c$. Thus, Eq 6 predicts what has been already experimentally demonstrated (3), that during a pressure relaxation test the pressure will decay exponentially from an initial constant value, P_i , to the critical turgor pressure, P_c , when P_c is constant. Furthermore, Cosgrove (2) has recently demonstrated a method to determine the value of the wall extensibility, θ , which also uses Eq 14.

(E) Determination of the Time-Dependent Turgor Pressure, $P(t)$, after an Instantaneous Change in the Magnitude of Biophysical and Biomechanical Parameters. The differential equation describing the behavior of the turgor pressure after an instantaneous change in the magnitude of pertinent biophysical and biomechanical parameters, is easily obtained using the new Growth Equation for the relative rate of volumetric expansion, Eq 6. We begin by noting that the rate of water uptake by the plant cell, with no transpiration, is described by the following (5, 7):

$$\dot{v} = dV/(V dt) = L(\Delta\pi - P) \tag{17}$$

where L is the hydraulic conductance and $\Delta\pi$ is the difference in osmotic pressure between the inside of the cell and the external medium (5, 7). Now it is apparent that Eq 17 and Eq 6 can be combined since the volume occupied by the cell contents and the volume of the cell wall chamber must be equal, and the rate of change of the respective volumes must also be equal. Thus, combining Eq 17 and Eq 6 by eliminating \dot{v} , and expanding and rearranging, we obtain:

$$dP/dt + \epsilon(\theta + L)P = \epsilon(L\Delta\pi + \theta P_c) \tag{18}$$

Applying the initial condition that at $t = 0$, $P = P_i$, the following solution is obtained for the simplest case, where ϵ , θ , P_c , L , and

$\Delta\pi$ are assumed to be constant:

$$P(t) = P_e + (P_i - P_e) \exp[-\epsilon(\theta + L)t] \quad (19)$$

where $P_e = (L \Delta\pi + P_c)/(\theta + L)$. It can be seen that at long times after an instantaneous change in parameter magnitude, $t = \infty$, the turgor pressure decays to a constant value, P_e . It should be noted that P_e is the same relationship derived for the turgor pressure for equilibrium (steady state) growth by Lockhart (5), and Ray *et al.* (7).

DISCUSSION

In this paper, the Growth Equation, for the relative rate of volumetric expansion of the cell wall chamber, as first derived by Lockhart (5) and subsequently by Ray *et al.* (7), is augmented to include 'elastic stretching' as well as irreversible wall extension. The augmented Growth Equation, Eq 6, is shown to reduce to the previously reported form for equilibrium growing, Eq 4, and to the form describing a turgid, nongrowing plant cell, Eq 7 and 8. Subsequently, the criterion for an accurate measurement of the volumetric elastic modulus, ϵ , for a growing plant cell was obtained, Eq 11, from this augmented Growth Equation.

Next the augmented Growth Equation was shown to reduce to an ordinary differential equation for the time-dependent turgor pressure, $P(t)$, when the condition for a pressure relaxation test is applied. The simplest solution to this differential equation (when ϵ , θ , and P_c are constant) predicts that the turgor pressure will decay exponentially, with a time constant of $1/\epsilon\theta$, from an initial value, P_i , to the critical turgor pressure, P_c . This analytical result draws support from the experimental results of Cosgrove *et al.* (3). They reported that the turgor pressure of pea plant cells (*Pisum sativum* L.) during a pressure relaxation test decays exponentially from an initial turgor pressure value to a constant value which they reason to be the critical turgor pressure. More recently, Cosgrove (2) also used this solution for a pressure relaxation test to determine the magnitude of the wall extensibility in pea stem cells.

Last, an equation describing $P(t)$, after instantaneous changes in pertinent biophysical and biomechanical parameters, was derived (Eq 19) using the equation describing the rate of water uptake and the augmented Growth Equation. It is shown that $P(t)$ decays exponentially from an initial value, P_i , to a constant value, P_e , after instantaneous changes in pertinent biophysical or biomechanical parameters occur. The time constant for this exponential decay is $1/\epsilon(\theta + L)$. It was further shown that the equilibrium pressure, P_e (achieved after the exponential decay), is described by the same relationship as the turgor pressure, P , previously obtained for equilibrium growth (5, 7).

In a previous paper, Cosgrove (1) also derived a differential equation for $P(t)$ from which a solution is obtained for the time-dependent turgor pressure after there occur instantaneous changes in pertinent biophysical and biomechanical parameters. In general, his derivation consists of differentiating, with respect to time, a linearized form of the differential equation defining

the volumetric elastic modulus and substituting the irreversible component of the wall extension for what is interpreted to be equivalent time derivatives of the volume. In Cosgrove's derivation, the distinction is made between two different volumes, that for the cell contents, V , and that for the cell wall chamber at 'incipient plasmolysis,' V_0 . The resulting differential equation is similar to that derived in this paper, Eq 18, but differs by a factor of V/V_0 . The inclusion of the extra factor, V/V_0 , results in a more complicated differential equation and a more complex solution. Cosgrove's final solution is similar in form and behavior to the solution obtained in this analysis (Eq 19) but differs in detail. A comparison of the two derivations reveals that the two are very similar in concept, but different in the mathematical extension of these concepts. A close analysis of Cosgrove's derivation suggests that the differences between the derived differential equations, and their respective solutions, stem from Cosgrove's using the linearized form of the differential equation which defines the volumetric elastic modulus, and differentiating the inclusive reference volume, V_0 , with respect to time in order to obtain a relationship which includes the elastic and irreversible components of cell wall extension. This procedure results in the introduction of the extra factor of V/V_0 into the differential equation.

The results presented in this paper argue that the use of the definition for the volumetric elastic modulus to derive a constitutive relationship that includes elastic stretching and irreversible extension is unnecessary. The use of the augmented Growth Equation eliminates the need to distinguish between two volumes, V and V_0 , and eliminates the extra ratio V/V_0 , thus simplifying the governing differential equation and its solution. Finally, it should be noted that although the solutions differ in detail both qualitatively and quantitatively, the quantitative difference may be small because for those plant cells in which the appropriate measurements have been made, it is found that the ratio V/V_0 is very near to unity.

Acknowledgments—The author wishes to thank Dr. Dan Cosgrove and Dr. John Trapp for their many fruitful and enjoyable discussions.

LITERATURE CITED

1. COSGROVE DJ 1981 Analysis of the dynamic and steady-state responses of growth rate and turgor pressure to changes in cell parameters. *Plant Physiol* 68: 1439-1446
2. COSGROVE, DJ 1985 Cell wall yield properties of growing tissue: evaluation by *in vivo* stress relaxation. *Plant Physiol* 78: 347-356
3. COSGROVE DJ, E VAN VOLKENBURGH, RE CLELAND 1984 Stress relaxation of cell walls and the yield threshold for growth: demonstration and measurement by micro-pressure probe and psychrometer techniques. *Planta* 162: 46-54
4. HUSKEN K, E STEUDLE, U ZIMMERMANN 1978 Pressure probe technique for measuring water relations of cells in higher plants. *Plant Physiol* 61: 158-163
5. LOCKHART JA 1965 An analysis of irreversible plant cell elongation. *J Theor Biol* 8: 264-275
6. PHILIP JR 1958 The osmotic cell, solute diffusibility, and the plant water economy. *Plant Physiol* 33: 264-271
7. RAY PM, PB GREEN, RE CLELAND 1972 Role of turgor in plant cell growth. *Nature* 239: 163-164
8. TAIZ L 1984 Plant cell expansion: regulation of cell wall mechanical properties. *Annu Rev Plant Physiol*: 585-657